

THE MICROSCOPIC FINDINGS
IN TWENTY-FOUR CASES OF
MALARIAL HEMOGLOBINU-
RIA.

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THE MICROSCOPIC FINDINGS IN TWENTY-FOUR CASES OF MALARIAL HEMOGLOBINURIA.*

BY ALBERT WOLDERT, M. D.,

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In the year 1902, I began to collect and observe a series of cases of malarial hemoglobinuria (black-water fever or black jaundice) with the intention of determining, if possible, the predisposing and exciting causes of this condition. My studies extended over a period of eight years, and during that time I made microscopical examinations of specimens obtained from twenty-four cases of black-water fever. This paper, therefore, is based upon eight years' work.

The method devised for carrying on my investigations was as follows:

1. To obtain complete history of the case, including the physical signs and symptoms presented.
2. Whether quinine, or any patent medicine, had been given previous to the onset of the paroxysms of hemoglobinuria.
3. The interval which elapsed between the time quinine or other alkaloid of cinchona was given, and the occurrence of hemoglobinuria.
4. The amount of quinine, or other alkaloid of cinchona, given (if any had been given) previous to the onset of blackwater fever.
5. Whether quinine aggravated the hemoglobinuria.
6. After the disappearance of hemoglobinuria, whether the administration of quinine produced a recurrence.

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7. Duration of the hemoglobinuria.
8. The period which elapsed before or after the first appearance of the paroxysm of hemoglobinuria and vomiting of bile.
9. The period which elapsed before or after the first appearance of the paroxysm of hemoglobinuria and the occurrence of jaundice.
10. Duration of the jaundice.
11. The period which elapsed before or after the administration of calomel and vomiting of bile.
12. The urine: (a) Chemical examination of urine, including the color, odor, reaction, specific gravity, presence or absence of glucose, presence or absence of indican, presence or absence of albumin and blood coloring matter, the amount of albumin, and in some cases the amount of uric acid and urea excreted daily. (b) Microscopical examination: The presence or absence of red blood corpuscles, presence or absence of ordinary tube casts, presence or absence of bodies resembling tube casts and made up of destroyed hemoglobin, presence or absence of ameba, or other microorganisms, both in the fresh and in the stained specimens of urine.
13. The blood: (a) Estimation of hemoglobin; (b) differential leucocyte count; (c) presence or absence of malarial parasites; (d) the resistant powers of the red blood corpuscles; (e) the presence or absence of other microorganisms than the malarial parasites in the blood.
14. The mortality.

STUDY OF THE BLOOD AND THE MALARIAL PARASITE.

In studying the blood in malarial hemoglobinuria, I have always found it best to use stained specimens. I would not advise microscopical examination of fresh specimens of blood in blackwater fever after the onset of hemoglobinuria occurs, for the reason that after the paroxysm begins, malarial parasites are generally so few in number that they may be entirely overlooked. In such cases a prolonged search is often required to find even one parasite.

The stain I used was a modification of the original Romanowsky method, modified first by Nocht, then by Ewing, then Hastings, and finally by myself. As finally made up, it was as follows: 1. A solution of eosin; 2, a solution of polychrome methylene blue; and, 3, a solution of methylene blue. These solutions were mixed when needed in certain definite proportions.

I prefer this stain above all others, because it is easily prepared when needed, keeps well, and all forms of the malarial parasites stain well with it, from the youngest merozoite and ring forms to the full grown parasites. The nuclear chromatin always stains a beautiful garnet color, the protoplasm a deep blue, the pigment granules black, the red corpuscles a deep pink, and the nuclei of the leucocytes a homogeneous purplish color. The azurophile granules also always show up well.

The percentage of malarial parasites found in the blood. In my series of twenty-four cases, the blood was examined in twenty-one. In all instances it was examined *after* the development of hemoglobinuria. I positively found malarial parasites in the blood in only two cases, while in two additional cases their presence was doubtful. In the two cases in which the parasites were positively found, both were of the estivoautumnal variety. In one of the cases the intracorpuseular ring form of the parasite was present, and the specimen showed the greatest number of parasites I have ever seen in any case of malarial fever. In this case perhaps one third of all the red corpuscles were infected, some red cells containing as many as three parasites each. This patient recovered. In the other case I found one ring form parasite within the cell, and a flattened merozoite attached to the outer rim of the corpuscle.

The positive results in this series of twenty-one cases examined would, therefore, be two per cent. In Panama, Whipple (1), in twelve cases of black-water fever, found malarial parasites present in

three cases, or twenty-five per cent.—all of them being of the estivoautumnal type.

Doubtless the small percentage of positive results in my series was due to the fact in every instance I examined the blood *after* the hemoglobinuria had begun. If the blood had been examined *before* the hemoglobinuria had occurred, the percentage of positive results would doubtless have been much larger. For instance, J. W. W. Stephens (2) reports from six different observers and says: "Of twenty-three cases on the day *before* hemoglobinuria developed, malarial parasites were found in twenty-two cases, or 95.6 per cent.; on the day hemoglobinuria occurred, out of sixty-three cases malarial parasites were found in thirty-nine cases, or 61.9 per cent.; and on the day *after* the onset of hemoglobinuria in sixty-four cases, eleven or 17.1 per cent.

Other statistics of blackwater fever have shown that on the day before the development of hemoglobinuria, malarial parasites were found in 61.9 per cent. of the cases, and on the day after development of hemoglobinuria, parasites were ~~found~~ in *about* eighty-three per cent. of the cases.

My results therefore confirm the work of others that *after* hemoglobinuria occurs *malarial parasites rapidly disappear from the peripheral blood.*

Hemoglobin reduction. As to the hemoglobin in certain cases, it seems quite remarkable that it may be reduced to such a low degree and yet support the life of the patient. In one of the cases of my series I found the hemoglobin was only ten per cent., confirmed by blood obtained from the ear and the finger, and yet that patient finally recovered. The blood picture in these cases, therefore, is always one of a profound degree of anemia.

The leucocytes. In the whole series (twenty-four cases) a differential leucocyte count was made in twelve cases; and so far as I am aware, it is the largest series of cases of blackwater fever in which a differential leucocyte count has been reported in

America. The counts were made for me, through the courtesy of my friend, Dr. L. Napoleon Boston.

The results of the count go to show that in blackwater fever there is a reduction in the normal percentage of polymorphonuclear leucocytes, occurring within a few hours of the time of onset of the paroxysm of hemoglobinuria and persisting for several days subsequently. How much one can attribute this reduction to the preexisting malarial fever and how much to the paroxysm of hemoglobinuria, I do not know.

In twenty counts, made in twelve cases, the polymorphonuclears averaged forty-nine per cent. of all leucocytes. In normal blood Cabot ⁽³⁾ gives the proportion of polymorphonuclears as ^{being} from sixty-two to seventy per cent.

In all cases of my series the large lymphocytes seemed to be increased from the time of onset of the paroxysm of hemoglobinuria to several days subsequently. It seems probable that this increase in the number of large lymphocytes indicates the malarial origin of the condition.

In twenty counts, made in the twelve cases, the large lymphocytes average twenty-seven per cent. of all leucocytes. In normal blood Cabot gives the proportion of large lymphocytes as being from four to eight per cent.

In nineteen counts, made in the twelve cases, the small lymphocytes averaged eighteen per cent. of all leucocytes. The small leucocytes, therefore, showed a slight reduction, since Cabot gives the normal proportion as being from twenty to thirty per cent.

An unusually high percentage of "mast" cells were present in two cases. A high percentage of myelocytes were present in five cases. Myelocytes, it should be recalled, are not present in normal blood, but are numerous in certain forms of primary anemias, such as myelogenous leukemia.

I found no other forms of microorganisms than the malarial parasites present in the blood in these cases of blackwater fever.

THE URINE.

In this series (twenty-four) of cases of black-water fever, the urine was examined in seventeen cases. Observation of the whole series proved that at the onset of the paroxysm of hemoglobinuria the urine within a few hours changed from the normal color to a dark red or port wine color, due to the contained hemoglobin. This red or port wine color, did not in a *single* instance depend upon the presence of red blood corpuscles. Not one of the cases in the entire series of seventeen examined, could be properly classed as one of hematuria. In eight out of the seventeen cases examined, I found that red blood corpuscles were *entirely* absent in the urine. In seven cases, and two doubtful instances in which red blood corpuscles were found, they were *never* present in sufficient quantity to give a red color to the urine. In some specimens I would occasionally find two or three red corpuscles. In one case I found nine or ten red cells present in each microscopic field. Free hemorrhage from the kidneys *never* occurred in any of my series of cases.

In all instances the reaction for blood coloring matter was obtained in the urine, when examined for, by placing a drop of urine on a piece of white muslin, and afterward adding a drop of freshly prepared tincture of guaiac, and a drop of ethereal extract of hydrogen peroxide, when a bluish zone would be produced at the point of contact.

The very large amount of albumin present in the urine, and the great amount of destroyed hemoglobin also present, indicates a high grade of dyscrasia. The suddenness with which the hemoglobin appears in the urine after a shaking chill, indicates that the excretion of blood coloring matter begins almost immediately after it has been thrown out of solution.

In most instances the urine was devoid of odor when first excreted, and in my experience seemed to require a longer time to undergo decomposition

than normal urine. I have kept specimens of the urine for several weeks, if not for months, apparently without a fetid odor occurring.

The reaction of the urine was in nearly every instance acid. Neither glucose nor indican were ever present. I have never found bile present in the urine in malarial hemoglobinuria, unless I had good reasons for believing that it had been contaminated with vomitus, which, in these cases, is often composed largely of bile.

In some instances the amount of albumin and blood coloring matter present after boiling amounted to fifty per cent. of the entire bulk of the urine, and in one instance, after boiling the urine, it solidified to such an extent that, upon inverting the test tube, only a few drops of clear, brownish urine ran out.

The relation of uric acid to urea in cases of malarial hemoglobinuria apparently varies considerably. In one of my cases the relation was one part uric acid to ten of urea; while in another case the relation was one part uric acid to sixty-three of urea.

In some specimens of urine, I repeatedly found certain round and concentrically arranged bodies, from ten to forty microns in diameter, often containing a darker brown dot or apparent nucleus. These bodies were doubtless inorganic in nature. They closely resembled organic bodies, but in my opinion were composed of uric acid. I frequently found bright, red crystals of hematoidin. I never found any special type of microorganism prevailing in the urine.

In several instances I found that disintegrated blood coloring matter (or destroyed hemoglobin), while under the cover slip, would arrange itself in the shape of tube casts. By touching the top of the cover slip gently with a needle, I found that I could at times make these loose particles of destroyed hemoglobin arrange themselves in the shape of tube casts. Barratt and Yorke (4) have found these

reddish granules entirely blocking the collecting tubules of the kidneys, and state that, "Suppression of the urine in blackwater is of mechanical origin, due to a blocking of the renal tubules"—an opinion with which it would be hard to disagree.

Of the seventeen cases in which the urine was examined, I found hyaline and granular casts, as ordinarily met with in nephritis, present in seven cases, and absent in ten cases. In nine cases out of the seventeen, I found bodies resembling tube casts, made up of disintegrated blood coloring matter, but what percentage of these bodies had been formed in the kidneys, and what percentage had been formed after being excreted from the body, I do not know.

After reviewing this series of cases, and in some instances watching them for several years after their recovery, I have never found in any instance evidence that nephritis developed as sequelæ of their attack of hemoglobinuria. During the paroxysm of hemoglobinuria, nephritis unquestionably occurs in many cases. I do not understand why nephritis is not a more frequent sequel of this condition.

THE CAUSE OF BLACKWATER FEVER.

In *every* one of these cases I found there was a history of malarial fever previous to the onset of the paroxysm of hemoglobinuria. In the twenty-four patients I found that six (or twenty five per cent.) had taken no quinine or other alkaloid of cinchona for a period of several days to a year previous to the onset of the hemoglobinuria. I proved the presence of cinchona alkaloids in the patent medicines taken in some cases, by chemical analysis and by obtaining the thalleioquin reaction. This large percentage of cases in which no quinine or other alkaloid of cinchona had been given previous to the development of hemoglobinuria, does not confirm entirely the observation of Koch (5) and Nocht and of others who entertain their views. Malarial fever was an invariable antecedent in the

production of the consequent hemoglobinuria. The *malarial parasite* is, therefore, *one* exciting cause of hemoglobinuria. On the other hand, we are forced to admit that during the course of malarial fever, and especially during the course of a chronic form (which need not necessarily be estivoautumnal infection), that quinine or other alkaloids of cinchona may and sometimes *do* cause a paroxysm of hemoglobinuria.

Of sixty patients with blackwater fever reported by Nocht, he states that fifty-eight had taken quinine, one other methylene blue, and still another antipyrine. Nocht further states that "each blackwater fever patient can tolerate a limited amount of quinine. The transgression *beyond* this amount produces blackwater fever." Koch concurred in these opinions of Nocht (6). Koch did not believe, nor does Nocht *now* believe, that quinine is the *only* cause of blackwater fever, but Koch (7) concurred in the views of Nocht that in *most cases* it is the quinine alone which brings on the attack. Nocht stated that the limit of the dose differed with each patient, and ranged from fifteen and a half grains to three twentieth grain. Ziemann had a case of blackwater fever in which the limit of the tolerance to quinine was three fortieths grain. In order to cure the predisposition to hemoglobinuria following quinine, Nocht found that it was best to begin giving the patient small doses of quinine and gradually to increase the dose until a tolerance has been established. He began with one sixth grain doses, and during prolonged periods gradually gave larger doses and in that way relieved the predisposition to blackwater fever.

In blackwater fever, the hemoglobin being so rapidly thrown out of solution, one might infer that hemolysis occurs in the blood stream. Nocht, however, is of the opinion that the hemolysis does not take place in the circulating blood, and that it does not depend upon a cooperation of hemolysin in the circulation with quinine, but that it is more proba-

ble that the hemolysis arises through the influence of the internal organs, such as the spleen, liver, or kidneys, without the influence of the hemolysin in the serum. Koch apparently concurred in this view of Nocht.

I found that by adding a drop of normal blood to a small amount of normal saline solution in a test tube and maintained for a few moments at the normal body temperature and containing a minute amount of quinine or other alkaloid of cinchona, that hemolysis occurred, these drugs dissolving out the hemoglobin and converting the red corpuscles into shadow or "ghost" cells, rendering the supernatant fluid reddish in color. However, it might be said that experiments have been made to prove that this hemolysis by quinine does not occur in the circulating blood.

The proof is overwhelming that quinine or other alkaloid of *cinchona* may be *one* exciting cause of blackwater fever (malarial hemoglobinuria, or black jaundice). Therefore, during the course of malarial fever (especially a chronic case in which quinine has been given), there appear to be two different agencies operating, either one of which has the power of producing or causing hemoglobinuria; and these two agencies or *causes* are: 1. The *malarial parasite*; and, 2, *quinine* or other alkaloid of cinchona.

It is, however, very evident that in those cases of malarial fever in which quinine has been given, and in which hemoglobinuria occurs, that it is the malarial parasite that has gone on before, crippling the red blood cells and paving the way for the secondary toxic or hemolytic effect of the quinine.

Having had practical experience of fourteen years in a district in east Texas, where malarial fever is more or less common, and having given this subject special study during that time, I feel that I am warranted in making the general assertion that the occurrence of malarial hemoglobinuria (blackwater fever) goes hand in hand with the

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prevalence of malarial fever, and when malarial fever disappears, blackwater fever also disappears.

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